The serotonin 1B receptor is required for some of the behavioral effects of psilocybin in mice

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Running title:

Abstract

Recent studies highlight the promising use of psychedelic therapies for psychiatric disorders, including depression. The persisting clinical effects of psychedelics such as psilocybin are commonly attributed to activation of the serotonin 2A receptor (5-HT2AR) based on its role in the acute hallucinatory effects. However, the active metabolite of psilocybin binds to many serotonin receptor subtypes, including the serotonin 1B receptor (5-HT1BR). Given the known role of 5-HT1BR in mediating depressive phenotypes and promoting neural plasticity, we hypothesized that it mediates the effects of psilocybin on neural activity and behavior. We first examined the acute neural response to psilocybin in mice lacking 5-HT1BR. We found that 5-HT1BR expression influenced brain-wide activity following psilocybin administration, measured by differences in the patterns of the immediate early gene c-Fos, across regions involved in emotional processing and cognitive function, including the amygdala and prefrontal cortex. Functionally, we demonstrated that 5-HT1BR mediates some of the acute and persisting behavioral effects of psilocybin. Although there was no effect of 5-HT1BR expression on the acute head twitch response, mice lacking 5-HT1BRs had attenuated hypolocomotion to psilocybin. We also measured the persisting effects of psilocybin on anhedonia and anxiety-like behavior using transgenic and pharmacological 5-HT1BR loss-of-function models and found that 5-HT1B is involved in mediating the decreased anhedonia and reduced anxiety-like behavior. Finally, using a network analysis, we identified neural circuits through which 5-H1BR may modulate the response to psilocybin. Overall, our research implicates the 5-HT1BR, a non-hallucinogenic serotonin receptor, as a mediator of the behavioral and neural effects of psilocybin in mice.

Introduction

In recent years, almost 10% of adults in the US meet the criteria for a mood disorder, and nearly 20% for an anxiety disorder (1). Despite the widespread use of selective serotonin reuptake inhibitors (SSRIs) as antidepressants and anxiolytics, these treatments require long-term administration and fail to provide relief for a significant proportion of patients (2). Developing novel therapeutic targets which include rapid-acting effective therapies is important for the treatment of depressive and anxiety disorders. Psychedelic compounds are emerging as potential therapeutic agents for treating psychiatric disorders. Growing evidence supports their efficacy in treating major depressive disorder (MDD) and other mood and anxiety disorders (3–8). Psilocybin is a serotonergic psychedelic found in *Psilocybe* mushrooms which is gaining traction as a promising therapeutic agent (9). Clinical studies report that one to two doses of psilocybin can produce antidepressant effects in MDD and treatment resistant depression patients that persist for weeks to months (5,7,10–14). However, the hallucinogenic properties of psilocybin limit its use for some patient populations and in everyday clinical practice. Uncovering the neural mechanisms responsible for the therapeutic benefits in preclinical studies is therefore crucial to developing other treatment strategies (15,16).

The persisting behavioral effects have been largely attributed to the activation of the 5-HT2A receptor, based on its well-documented role in mediating the acute hallucinogenic properties of serotonergic psychedelics (17–21). However, there is conflicting evidence emerging from preclinical studies on the necessity of 5-HT2AR in the behavioral effects, especially related to depressive behavior using pharmacological and genetic loss-of-function models (22–28). Some recent studies suggest that these behavioral and neural effects may be independent of 5-HT2AR activation (23,27). Psilocybin's capacity to drive neuroplastic changes has been demonstrated through structural changes in dendritic spines, including increased spine density and size in the prefrontal cortex and synaptic changes in the hippocampus (29) —all alterations which may remain intact with blockade of psilocybin binding to 5-HT2A receptors (23,30). Recent studies also implicate the neurotrophin receptor TrkB and brain-derived neurotrophic factor (BDNF) signaling in mediating psilocybin-induced synaptic plasticity, independent of 5-HT2ARs (31).

Psilocybin's active metabolite, psilocin, binds with high affinity to many serotonin receptor subtypes, not just 5-HT2A, and its polypharmacology is likely important for its clinical efficacy (32). One receptor besides 5-HT2A that binds psilocybin and may be important for the effects of psilocybin on behavior is the serotonin 1B receptor (5-HT1BR). As an inhibitory Gi/o-coupled G protein-coupled receptor (GPCR) that inhibits neurotransmitter release at axon terminals, it has been implicated in a number of plasticity mechanisms including long-term potentiation of cortical inputs to the hippocampus, and long-term depression of corticostriatal synapses (33,34). 5-HT1BRs have also been linked to increased anxiety and depressive behaviors (35–37). Finally, 5-HT1BR has been implicated in mediating the effects of other psychoactive substances including MDMA and Ketamine (38–42). These data suggest that 5-HT1B could be a critical mediator of psilocybin's persisting therapeutic effects.

Here, we hypothesize that psilocybin modulates depressive-like behaviors through 5-HT1B receptor activation. Using a genetic and pharmacological loss-of-function models,

we sought to investigate the role of 5-HT1B in neural and behavioral effects of psilocybin in mice. We assessed the role of 5-HT1BR in modulating the acute drug effects of psilocybin on brain-wide neural activity, head twitch, and locomotor behavior. We also investigated the impact of 5-HT1BR on the long-term behavioral effects of psilocybin on anxiety and anhedonia phenotypes, key features of depressive disorders. Our findings suggest that the 5-HT1BR influences brain-wide neural changes following psilocybin administration and is necessary for its enduring antidepressant-like effects in mice

Materials and Methods

Animals. Mice were bred in the Dartmouth College vivarium and weaned at postnatal day (PN) 21 into cages of 2–4 same-sex littermates. Cohorts of mice lacking 5-HT1BR expression and littermate controls were generated by crossing female homozygous floxed-tetO1B mice to male homozygous floxed-tetO1B mice with the βActin-tTS transgene (tetO1B+/+ females crossed to tetO1B+/+::βActin-tTS + males), as previously reported (43). N=27 (11 males, 16 females) were used in the c-Fos study. N=52 mice (31 females, 21 males) were used to assess the acute behavioral effects of psilocybin. N=155 (60 males, 95 females) were used to assess persisting behavioral effects of psilocybin following chronic corticosterone administration. N=164 mice (61 females, 103 males) were used to assess persisting behavioral effects of psilocybin following chronic behavioral despair. Mice were run in 3–4 experimental cohorts, and all treatment conditions were represented within each cohort. N = 43 female mice were used for the antagonist experiment. For the adult knockout experiment (N=30), temporal-specific knockdown was achieved by withdrawal of doxycycline (Product # F5545, Bio-Serv, Flemington, NJ) at PN60 from mice following administration to the dam throughout gestation and nursing, and to the mice following weaning. As previously reported, this results in normal levels of 5-HT1BR expression through development and knockout in adulthood (43). Following PN120, mice were then administered chronic corticosterone administration and persisting behavioral effects of psilocybin were examined. All mice were maintained on a 12:12 light-dark cycle and on *ad libitum* chow and water except as noted below for treatments and behavioral testing. Mice were between ages 3-9 months old at time of testing. All procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee.

Drugs and treatments. Psilocybin (Cat # 14041, Cayman Chemical), or saline vehicle, was injected intraperitoneally (i.p.) at the dose of 5mg/kg diluted in saline solution (0.9% NaCl) at a volume of 10ml/kg. The dose of 5mg/kg was chosen based on our pilot experiments in this strain of mice that induced post-acute behavioral changes. Although the dose is on the higher side of the usual range of 1-5mg/kg commonly used in rodents, is in line with other reports assessing behavioral effects of psilocybin in mice with doses over 4mg/kg (44–46). For antagonist experiments, 0.9% saline vehicle, or the serotonin 5-HT1B/1D receptor antagonist GR127935 (CAT#508014; Sigma-Aldrich) was injected i.p. at 10mg/kg (in 0.9% saline at 10ml/kg volume) 30 minutes prior to psilocybin or saline vehicle administration, as previously used for pretreatment in behavioral pharmacology studies to target 5-HT1B/D receptors (47–50).

Chronic corticosterone administration. Corticosterone (35 µg/mL; Cat # 2505, Sigma-Aldrich) was dissolved in 0.45% beta-cyclodextrin (Cat # 4767, Sigma-Aldrich) in drinking water, and delivered *ad libitum* in opaque bottles for 4 weeks. This paradigm produces a corticosterone dosing of approximately 9.5 mg/kg/day (51). In non-stressed

mice, only 0.45% beta-cyclodextrin was dissolved in drinking water, and delivered *ad libitum* in opaque bottles for 4 weeks.

Chronic behavioral despair model. As an alternative to chronic corticosterone administration, a separate group of male mice were subjected to repeated stress exposures via five forced swim sessions. Each mouse was placed in a 2-liter beaker containing water (22-25ºC) for 10 minutes daily for five consecutive days. Mice remained in their home cages between sessions. Mice were injected with psilocybin (1mg/kg, i.p) or saline vehicle (10 ml/kg) 48 hours after the last swim session.

Behavioral tests.

Head Twitch Response and Locomotion. Head twitches and locomotion were analyzed in a subset of mice after the administration of either vehicle or psilocybin (5mg/kg, i.p) in both control and mice lacking 5-HT1BR. Immediately following injections, mice were placed in an open field, and video recording was initiated with a camera positioned directly above the arena for 1 hour. Three experimenters scored the first 15 minutes of activity for head twitches while blinded to treatment, noting the total number of and time stamp of each head twitch to compare across experimenters. Locomotion was scored with EthoVision software.

Gustometer. A Davis Rig 16-bottle Gustometer (Med Associates MED-DAV-160 M) was used to test the effects of psilocybin and 5-HT1BR expression on hedonic responding. Mice were water-restricted for 5 days of initial training before being put on corticosterone. On day 1 of training, mice were placed in the gustometer as a cage and with free access to 5% sucrose for 15 minutes. On day 2, mice were placed individually with free access to 5% sucrose for 15 minutes. On day 3, mice were placed individually with access to 5% and 10% sucrose with tubes alternating for 30 minutes. Mice were then food restricted for 2 days before being individually returned to the gustometer for 30 minutes with access to water, 2% sucrose, 4% sucrose, 6% sucrose, 8% sucrose, and 10% sucrose for a final training day using 6 bottle paradigm. Each concentration was randomly presented for a maximum of 120 seconds at a time before switching to another tube. Total licks at each tube were recorded using a capacitance-based system. Mice that had less than 300 licks during the final training session were excluded from the analysis. After the final training session, mice were put on corticosterone. The 6 bottle paradigm was then repeated 24 hours after drug administration for the experimental gustometer test. Each mouse was weighed before the 6 bottle paradigm and data were normalized by weight. After the experimental test, mice were allowed food that day until 24 hours before the time of the novelty-suppressed feeding test (about ~1h30).

Novelty Suppressed Feeding (NSF). The NSF assay was conducted in a rodent transport container (20' x 16') with the floor covered with approximately 2-4cm of corn bedding to serve as an arena. Thirty minutes before testing, mice were placed individually into holding cages. At the time of testing, a single pellet of food was placed on a white platform (~4' diameter) in the center of the arena. The anxiogenic environment was produced by placing a lamp with high luminosity (~120 lux) above the center of the arena. Each mouse was placed in the same corner of the arena, facing the wall, and a stopwatch was immediately started. The latency to eat, defined as the mouse biting the pellet, was recorded over a session of 5 minutes. Immediately after a bite, the pellet was removed from the arena. To control for hunger, mice were immediately placed

208 in their homecage after the testing session with a new food pellet, and the stopwatch
209 was started again to measure latency to eat within their homecage. Animals that did not
210 bite during the testing session were attributed the maximum value (300 seconds).
211 Animals were placed back in their homecage with food and water ad libitum once all
212 cage mates were tested. The NSF was performed 24 hours after the gustometer in the
213 corticosterone stress paradigm, and 48 hours after the EPM in the chronic behavioral
214 despair paradigm.

215 *Elevated Plus Maze (EPM)*. Mice were placed in the center facing a closed arm and
216 videotaped while allowed to explore the maze undisturbed for 6 minutes. The maze has
217 four arms (35cm long x 5cm wide) that were 60cm above the ground. The two opposite
218 closed arms had 20cm high walls, while the two opposing open arms had a 1cm lip.
219 Behavior was scored using EthoVision software for the number of entries and time spent
220 in each arm. Mice were excluded from the analysis as outliers if the number of open arm
221 entries was greater than or less than two standard deviations from the mean. The EPM
222 was performed 24 hours after the NSF in the corticosterone stress paradigm, and 24
223 hours after the gustometer in the chronic behavioral despair paradigm.

224

225 **c-Fos Whole Brain Analysis**

226 *Brain samples.* A set of male and female tTS- (N=14) and tTS+ (N=14) mice not
227 maintained on doxycycline was used to perform a whole brain c-Fos analysis following
228 vehicle or psilocybin (5mg/kg, i.p) administration. Brain-wide c-Fos expression was
229 induced during the acute phase of psilocybin administration. Mice were injected with
230 either vehicle or psilocybin (5mg/kg), and then placed back in their home cage for 2h,
231 before perfused with 0.1 M PBS followed by 4% formaldehyde. Brains were extracted
232 and postfixed for 24 hours in 4% formaldehyde before being cryoprotected in 30%
233 sucrose. Brains were then sectioned in a coronal plane with a thickness of 40um on a
234 cryostat (Leica Biosystems) and stored in 0.1 M PBS at 4ºC in 24 well plates. About 20
235 to 30 sections per brain were selected for staining. One brain in the saline-WT group
236 was eliminated due to technical issues with tissue sections prior to processing.

237 *Tissue processing*. Sections were first washed three times in PBS-T (0.1%) for 10
238 minutes. Sections were then blocked for 1h in 2% Normal Donkey Serum (#NC9624464,
239 Fisher) in PBS-Triton (0.1%). Sections were then incubated in primary antibody (rb anti-
240 cfos Rabbit Recombinant Monoclonal c-Fos antibody, ab214672) at 1:1000 in blocking
241 solution overnight at 4ºC. The following day, sections were washed 3 times again in
242 PBS-T (0.1%). Sections were then incubated in secondary antibody Alexa Fluor 488
243 anti-rabbit (ab150073, Abcam) at 1:250 and DAPI at dilution 1:10,000 in PBT (0.15) for 2
244 hours away from light. Sections were then washed again three times and mounted.
245 Once dry, the slides were cover slipped with ProLong Gold. Whole sections were then
246 imaged on Keyence BZ-X800 microscope with a 10x objective. Fluorophores were
247 imaged with DAPI and GFP filter cubes.

248 *Segmentation of fluorescent labels and quantification*. Cells expressing a c-Fos label
249 were segmented using the machine learning-based pixel and object classification
250 program, *Ilastik*, as previously described in Berg et al., 2019. *Ilastik* was also used to
251 generate binary images of the segmented c-Fos labels. Registration and quantification of

histological images on a brain-wide scale were performed using the open-source atlas registration tool FASTMAP, as previously described in (52). Regions that were not adequately aligned or identified on stained sections were left out of the overall analysis.

Statistical Analyses

All behavioral analyses were performed using GraphPad Prism (v.10.2.2) or RStudio (v.2023.06.1+524). The threshold of statistical significance was set at 0.05. Data were analyzed using one-, two-, and three- ANOVAs, linear mixed models, generalized linear models, and t-tests using GraphPad Prism and RStudio, with Tukey's correction for multiple comparisons when appropriate. Area under the curve for locomotion was calculated using the trapz function in RStudio. Functional connectivity analyses were performed on a collection of 30 regions based on our interest and ability to reliably and consistently delineate these regions using a DAPI stained reference image. A psilocybin index was generated by subtracting average total c-FOS counts. The density of regional c-Fos expression was cross-correlated within each group to generate pairwise correlation matrices, and difference matrices were obtained by subtracting coefficients within each element. To quantify psilocybin-induced changes in network synchrony within each genotype, we extracted the upper triangle to obtain pairwise correlation changes. We then performed a one-sample-t-test on each vector to determine whether the mean change in correlation significantly differed from zero within each genotype. The similarity and strength of matrices were assessed using nonparametric permutation testing Monte Carlo and Spearman correlation to generate rho and p values. For network analysis, we performed a fisher r-to-z transformation on correlation and selected significant edges at 95%, 90%, 85%, and 80% confidence intervals.

Results

Psilocybin-induced changes in neural activity require 5-HT1BR expression

To investigate how psilocybin changes brain-wide neural activity, and whether 5-HT1BR expression influences those changes, we performed a whole-brain c-Fos analysis on male and female mice lacking the 5-HT1BR and their littermate controls. Brains were collected 2h following a single injection of psilocybin (5mg/kg) or saline vehicle, and we found that the number of c-Fos+ cells was relatively consistent across animals within brain regions (Fig 1A). Overall, there was a significant effect of psilocybin on neural activity measured by c-Fos staining (main effect of psilocybin: $F(1,23)=5.708$, $p=0.026$). Interestingly, the neural activity was significantly influenced by 5-HT1BR expression (genotype by drug interaction: $F(1, 23)=5.986$, $p=0.023$), though the pattern varied by brain region (Fig S1; genotype by drug by region interaction: $F(28, 628) = 2.352$, $p=0.0001$). Some brain regions showed significant effects of psilocybin regardless of 5-HT1BR expression, such as the somatosensory cortex and the claustrum (CLA) which both express high levels of 5-HT2ARs (Fig 1B, main effect of drug on CLA: $F(1, 22)=21.74$, $p=0.0001$). There were also select brain regions, such as the basolateral amygdala (BLA) which showed differential baseline c-Fos activity based on genotype (main effect of genotype: $F(1, 22) = 4.832$, $p = 0.039$) which potentially drove the differential effect of psilocybin between genotypes (genotype by drug interaction: $F(1, 22) = 11.13$, $p = 0.003$). Most interestingly, quite a few other brain regions showed an effect of 5-HT1BR expression on the c-Fos response to psilocybin including prefrontal cortical areas and the central amygdala (CEA) which showed increased c-Fos in control,

but not 5-HT1BR KO mice (drug by genotype interaction for CEA: F(1, 23)=8.084, p=0.009; controls: p=0.0002, 5-HT1BR KOs: p=0.669). The globus pallidus showed a similar pattern with increased c-Fos expression in controls, but not 5-HT1BR KO mice (drug by genotype interaction for GP: F(1, 22)=4.954, p=0.037; controls: p=0.003; 5-HT1BR KO: p=0.829). Importantly, these regions express high levels of 5-HT1BR protein (Fig S2; (53,54), and may be key sites for psilocybin acting via these receptors to influence neural activity. Overall, psilocybin differentially influenced neural activity throughout the brain leading to both increases and decreases depending on the brain area, and these changes in neural activity were differentially influenced by 5-HT1BR expression (Fig 1C). This points to a role for the 5-HT1BR to mediate the behavioral effects of psilocybin in rodents.

5-HT1BR may influence some of the acute behavioral effects of psilocybin

To investigate the functional effect of 5-HT1BR activation on the acute behavioral response to psilocybin, we measured the head twitch and locomotor response to psilocybin in male and female control and 5-HT1B KO mice. Mice treated with psilocybin demonstrated significantly more head twitch responses than mice treated with saline (Fig 2A; main effect of psilocybin: F (1, 28) = 312.3). However, there was no significant difference in the number of head twitches between genotypes (main effect of genotype: F (1, 28) = 0.4982), indicating that psilocybin robustly induces head twitches in mice independent of 5-HT1BR. This is consistent with previous research implicating 5-HT2AR activation in this acute response to psilocybin (55,56), and demonstration of normal levels of 5-HT2AR expression in mice lacking 5-HT1BRs (57). We also analyzed the locomotor response to psilocybin in mice lacking 5-HT1BR expression, and found that unlike the head twitch response, 5-HT1BR may be involved in the acute hypolocomotor effects (Fig 2B). Specifically, psilocybin significantly decreased locomotion in the 30min following administration (F(1, 48)=8.18, p<0.0063), without effects on velocity (Fig S3A). This effect seemed attenuated in mice lacking 5-HT1BR, however this interaction didn't reach statistical significance (genotype by psilocybin interaction F(5, 240)=2.00, p=0.0791). Using AUC values for planned comparisons within genotype (Fig 2C), there were significant reductions in locomotor activity following psilocybin in control (t_{28}=2.92, p=0.0068), but not in 5-HT1BR KO mice (t_{20}=1.26, p=0.16). Overall, these data show that 5-HT1BR could partially mediate some acute behavioral responses to psilocybin, though not the classic head twitch response.

5-HT1BR is required for antidepressant-like effects of psilocybin in female mice treated with corticosterone

Given that there are long lasting behavioral effects of a single administration of psilocybin in humans, we next wanted to examine the role of the 5-HT1BR in the persisting behavioral effects in mice. We used two paradigms to model stress-induced anhedonia and anxiety-like behaviors. First, we administered chronic corticosterone in the drinking water for four weeks to induce a stress-phenotype to measure antidepressant-like effects of psilocybin (Fig 3A), which did not influence the acute responses to psilocybin (Fig S3B-C). Interestingly, we tested mice lacking 5-HT1BR expression and littermate controls in a series of behavioral tests that evaluated anhedonia and anxiety-like behaviors, performed 24-72h after a single i.p. injection of either vehicle or 5mg/kg psilocybin. In female mice, psilocybin significantly increased hedonic responding in the gustometer as measured by increased licking for sucrose (Fig

345 3B; treatment by sucrose concentration: F(2,384) = 5.71, p = 0.004). There was also a
346 significant effect of genotype on licking across sucrose concentrations (F(1, 384) = 9.12,
347 p = 0.003) which is consistent with the baseline differences between genotypes that we
348 have previously reported (58). Though the interaction of genotype and treatment did not
349 reach statistical significance (F(2, 384)=2.53, p=0.081), because there were baseline
350 differences, we compared the effect of psilocybin within each genotype. While control
351 mice showed a significant effect of treatment on sucrose consumption (Fig 3B; F (2, 42)
352 =6.09, p=0.005), there was no effect in female mice lacking 5-HT1BR (F(2, 30) = 0.73,
353 p=0.489). Looking specifically at consumption of the highest concentration of sucrose in
354 control mice (Fig 3C; main effect of treatment F(2, 72)=5.66, p=0.005), psilocybin
355 increases consumption compared to saline treated mice (p=0.012), restoring it back to
356 non-cort baseline levels (p=0.266). In 5-HT1BR KO mice, although cort decreased
357 sucrose consumption (no cort/saline vs cort/saline: p=0.030), psilocybin did not
358 significantly increase the consumption (cort/saline vs cort/psilocybin: p=0.549). Overall,
359 these data suggest that psilocybin may influence anhedonia in mice through actions at
360 the 5-HT1BR.

361 Expression of 5-HT1BR also significantly modulated the anxiolytic response to psilocybin
362 in female mice. First, we measured anxiety-like behavior in the conflict-based novelty-
363 suppressed feeding (NSF) assay 48h following psilocybin administration. Interestingly,
364 there was an anxiolytic response to psilocybin that was significantly blunted in the
365 absence of 5-HT1BR expression (genotype by drug interaction: F(2,72) = 2.99, p =
366 0.049). Specifically, control mice injected with psilocybin showed reduced latency to eat
367 compared to saline-treated mice (Fig. 3D, χ^2 = 9.62, p = 0.002), while we saw no
368 significant effect in the 5-HT1BR KO mice (Fig. 3D, χ^2 = 1.85, p = 0.174). There was also
369 a main effect of genotype independent of treatment (F(1,72)=7.29, p=0.009), suggesting
370 that there are again baseline effects of brain-wide, life-long absence of 5-HT1BR
371 expression on anxiety-like behavior. There were no significant effects of genotype or
372 drug treatment in the home cage condition suggesting that the effects of psilocybin were
373 on anxiety behavior rather than feeding behavior (Fig S4A). We also tested mice in the
374 elevated plus maze (EPM), as a second measure of anxiety-like behavior, 72h following
375 psilocybin administration. We again found that psilocybin-induced decreases in anxiety
376 behavior that were dependent on 5-HT1BR expression (Fig. 3E; main effect of drug by
377 genotype (F(1, 51) = 4.080, p = 0.0487). Psilocybin administration significantly
378 decreased anxiety in female control mice, as measured by increased entries in open
379 arms (p=0.01), but not in mice lacking 5-HT1BR (p = 0.732). This was also somewhat
380 reflected in the time spent in open arms of the EPM (Fig S4B), and was not due to any
381 persisting changes in overall locomotion (Fig S4C). Overall, convergent evidence from
382 two conflict-based assays shows that psilocybin's persisting anxiolytic response requires
383 5-HT1BR. Overall, these behavioral results indicate that in female mice, psilocybin
384 produces an antidepressant-like phenotype in the days following administration which
385 we measured as decreased anhedonia in the gustometer and decreased anxiety-like
386 behaviors in the NSF and EPM. Interestingly, these behavioral effects were attenuated
387 in the absence of 5-HT1BR expression, suggesting that the antidepressant-like effects of
388 psilocybin are dependent on 5-HT1BR following chronic corticosterone administration in
389 female mice.

390 Interestingly, in male mice, we were unable to find any behavioral effect of psilocybin
391 following chronic corticosterone treatment, which is consistent with previous findings
392 (27). Specifically, psilocybin had no effect on hedonic responding in the gustometer as

measured by licking for various sucrose concentrations (Fig 3F; $F(2, 230.4)=2.14$, $p=0.12$). Similar to the females, we again saw an effect of genotype on licking over the sucrose concentrations in males ($F(1, 294)=11.36$, $p<0.001$), as previously reported (58), however there was no significant effect of treatment ($F2, 294)=2.37$, $p=0.1$). Analyzed within each genotype, there was no effect of psilocybin in either 5-HT1BR KOs or controls ($F(2,23)=0.60$, $p=0.55$ for controls, $F(2, 31)=1.1$, $p=0.34$ for KOs). Similar patterns were seen when looking at consumption of the highest concentration of sucrose (Fig 3G). There was a significant genotype effect, but no effect of treatment ($F(1, 54)=5.3$, $p=0.028$ for genotype, $F(2, 54)=1.3$, $p=0.28$ for treatment).

We saw a similar trend for males in two tests of anxiety-like behavior – there were effects of cort treatment and of genotype, but no significant effects of psilocybin on these measures. In the NSF test (Figs 3H and S4D) 5-HT1BR KOs showed reduced anxiety-like behavior ($F(1, 55)=18.12$, $p<0.0001$), but again there were no effects of psilocybin, despite cort treatment increasing anxiety-like behavior (main effect of treatment: $F(2,55)=5.92$, $p=0.005$, veh/saline vs cort/saline: $\chi_1 = 6.72$, $p=0.006$ for controls, $\chi_1 = 11.13$, $p=0.001$ for KOs; cort/saline vs cort/psilocybin: $\chi_1 = 0.50$, $p=0.48$ for controls, $\chi_1 = 1.66$, 0.24 for KOs). In the EPM (Figs 3I, 4SE-F), there were no effects of genotype on the number of entries into the open arms in males ($F(1,54)=2.22$, $p=0.14$). There was a significant effect of treatment ($F(2, 54)=12.64$, $p<0.0001$), though this effect was driven by the effect of cort (veh/saline vs cort/saline: $p=0.0007$), rather than psilocybin (cort/saline vs cort/psilocybin: $p=0.57$). Overall, although genotype and cort had some behavioral effects in males, psilocybin consistently did not influence anhedonia and anxiety-like behavior.

Acknowledging potential compensatory effects of the absence of 5-HT1BR throughout development, we used two approaches to address this alternative interpretation of the psilocybin effect in females. First, we used an adult-knockdown approach, in which mice were reared with normal expression of 5-HT1BRs through PN60 via administration of doxycycline to block tTS-mediated knockdown (as previously reported and validated (58)). Following withdrawal of doxycycline as adults, mice lacking 5-HT1BR expression were tested for the persisting behavioral effects of psilocybin during chronic corticosterone administration in the elevated plus maze (Fig 4A). Though limited in number, a planned comparison shows no significant effect of psilocybin in adult knockdown mice ($t_{14}=0.26$, $p=0.80$). Though the interpretation of this result is limited by an effect in controls that was only trending toward significance ($t12=1.461$, $p=0.11$), a lack of effect in the adult knockdown suggests that any developmental compensation was likely not responsible for the lack of effects seen in the whole-brain whole-life knockouts. This effect was mirrored in the time spent in the open arms (Fig S5A), and was not due to differences in locomotion (Fig S5B). Additionally, to investigate if the behavioral effects in the constitutive 5-HT1BR KO were due to activation of the 5-HT1BR during the acute phase of psilocybin, and to attempt to circumvent genotype differences seen in some of the behavioral tests, we performed another experiment in which we pharmacologically blocked 5-HT1BR with pre-treatment with a 5-HT1B/1D receptor antagonist, GR127935, during psilocybin administration in control mice. Psilocybin again decreased anxiety-like behavior as measured by increased open-arm entries as a function of 5-HT1BR activation (Fig. 4B, pretreatment x psilocybin interaction: $F(1, 48)=4.30$, $p=0.044$). Specifically, in the absence of any pretreatment, psilocybin increased open arm entries ($p=0.0084$), however when pre-treated with GR127935, the effect of psilocybin was blocked ($p=0.89$). Measures of open arm duration or locomotion

were not significantly influenced by treatment (Fig S5C-D). Overall, these results suggests that blockade of 5-HT1BR during the acute psilocybin response may be sufficient to block the post-acute anxiolytic effects of psilocybin seen in the EPM.

5-HT1BR mediates the anxiolytic effects of psilocybin in a behavioral despair model in both female and male mice

We used a second stress paradigm to confirm the results seen in the female mice in the chronic cort model, and because the chronic cort model was not effective to measure the persisting behavioral effect of psilocybin in male mice. Using a chronic behavioral despair paradigm implemented through repeated forced swim stress, we examined the effect of the absence of 5-HT1BR on the behavioral effects of psilocybin in mice (Fig 5A). Interestingly, in this paradigm we were able to elicit an effect of psilocybin in both sexes. There were significant effects of treatment on consumption across the sucrose concentrations (Fig 5B; treatment x concentration: $F(2, 538)=3.63$, $p=0.027$). Given that we saw a baseline genotype effect again (genotype x concentration: $F(1, 538)=10.29$, $p=0.001$; genotype x treatment x concentration $F(2, 538)=2.41$, $p=0.09$), we analyzed the treatment effects within each genotype separately. In control mice, there was a significant effect of treatment on sucrose consumption (treatment x concentration: $F(10, 330)=2.87$, $p=0.002$), which was largely driven by a increase in consumption of 10% sucrose following psilocybin compared to saline treatment (Fig 5C; $p=0.009$). Though there were no significant sex differences ($F(1,488)=0.86$, $p=0.35$) and both sexes showed similar trends, the effects of psilocybin in females may be greater (Figs S6A-D). On the other hand, there was no effect of treatment in 5-HT1BR KO mice ($F(10, 190)=0.75$, $p=0.68$), suggesting that stress and/or psilocybin don't significantly influence hedonic responding in the absence of 5-HT1BR expression in mice.

In the EPM, there was a significant effect of genotype on the anxiolytic effect of psilocybin (Fig 5D; interaction of genotype and treatment: $F(2, 155)=4.22$, $p=0.016$). In control mice, stress decreased entries into the open arms ($p<0.0005$), and psilocybin rescued this effect ($p=0.03$). Interestingly, in the mice lacking 5-HT1BR expression, psilocybin had no effect on entries into the open arms ($p=0.99$), though stress also had no effect in this strain ($p=0.84$). This pattern was replicated in the duration of time spent in open arms of the EPM (Fig S6E-G). There were also no effects on locomotion generally in the EPM (Fig S6H-J), and no significant effects of sex on the EPM behavior (genotype x treatment x sex: $F(2, 155)=0.146$, $p=0.86$).

In a second measure of anxiety behavior in the NSF assay, psilocybin also decreased anxiety-like behavior as measured by latency to eat (Fig 5E, $X_5=31.40$, $p<0.0001$). There were again some baseline differences between genotypes, but importantly within control mice, psilocybin decreased the latency to eat (stress/saline vs stress/psilocybin: $X_1=6.41$, $p=0.011$), but had no significant effect in 5-HT1BR KO mice ($X_1=1.70$, $p=0.19$). There were again no effects of sex on the treatment effects (sex by treatment: $F(2, 104)=0.14$, 0.87). There were no effects of genotype or psilocybin on latency to eat in the homecage suggesting that hunger was not a cause of the differences in behavior (Fig S6K-M).

485 **Neural circuits impacted by psilocybin are influenced by 5-HT1BR expression**

486 To identify the neural circuits through which 5-HT1BR may mediate the effects of
487 psilocybin on behavior, we performed a network analysis on neural activity following
488 psilocybin to probe functional connectivity. Returning to the c-Fos data (shown in Fig 1),
489 we generated pairwise correlation matrices of brain region activity as measured by
490 number of c-Fos+ cells to determine how the activity of brain regions vary together. A
491 difference matrix for each genotype, representing the change in correlated activity
492 following psilocybin administration, illustrates how the functional connectivity throughout
493 the brain is changed by psilocybin and differs based on 5-HT1BR expression (Fig. 6A).
494 Comparing these two correlation matrices reveals statistically distinct patterns between
495 control and 5-HT1BR KO mice (Fig 6A; rho = -0.01, p = 0.80). Control mice showed
496 more desynchrony following psilocybin compared to saline (t = -5.336 with p<0.0001).
497 Interestingly, this is reversed in mice lacking 5-HT1BR (t=+5.083 p < 0.0001 for KOs),
498 suggesting that neural activity is more positively correlated across regions following
499 psilocybin treatment. We then examined which psilocybin-responsive networks were
500 most influenced by 5-HT1BR expression and found significant effects of 5-HT1BR on
501 correlated activity between the central amygdala and the anterior cingulate cortex
502 (z=2.00, p=0.05). Widening the confidence interval, we found suggestive differences in
503 the correlated activity of the central amygdala with the claustrum, amongst amygdalar
504 regions, and between the globus pallidum and lateral amygdala and anterior cingulate
505 cortex (Fig 6B). Overall, this network analysis shows that 5-HT1BR expression
506 significantly influences neural circuits that are relevant to the effects of psilocybin on
507 cognition and mood. Furthermore, it points to potential neural circuits through which 5-
508 HT1BR may influence some of the persisting effects of psilocybin.

509

510 **Discussion**

511

512 Our results highlight a critical role for 5-HT1BR in the response to psilocybin in mice. We
513 found that the absence of this non-hallucinogenic receptor impacts the neural response
514 to psilocybin, as well as the acute and persisting behavioral effects. Using c-Fos as a
515 marker of neural activity, we find increased activity in many regions throughout the brain
516 including in sensory and prefrontal cortices, claustrum, amygdala, hypothalamus, and
517 basal ganglia, which is consistent with previous reports (22,59–61). Interestingly, we saw
518 that mice lacking expression of 5-HT1BR have notable differences in the pattern of
519 neural activity following psilocybin administration, including in prefrontal, hippocampal,
520 and amygdalar areas, all brain regions that express high levels of 5-HT1BR.
521 Functionally, a lack of 5-HT1BR had no effect on the head twitch response, though it did
522 attenuate the acute hypolocomotor effects of psilocybin. Importantly, there were also
523 effects of 5-HT1BR on the persisting behavioral effects of psilocybin seen by the
524 amelioration of stress-induced anhedonia and anxiety-like behaviors. Overall, this shows
525 that 5-HT1BR expression is involved in mediating some the neural and behavioral
526 effects of psilocybin..

527 Though we find that 5-HT1BR is required for the persisting effects of psilocybin, our data
528 do not address its sufficiency. The profound neural and behavioral effects of psilocybin
529 are likely supported by its polypharmacology at multiple serotonin receptors (32).
530 Although there is some evidence questioning the necessity of the 5-HT2AR in the
531 behavioral effects of psilocybin in mice (23,26), there are mixed findings on this topic

532 with other reports showing that 5-HT2AR expression is necessary for the anti-
533 depressant effects of psilocybin (22). Tangential to this debate, our results show that
534 acute activation of the 5-HT2A receptor by psilocybin is insufficient to rescue stress-
535 induced behaviors in the absence of the 5-HT1BR.

536 There are many lines of evidence linking the 5-HT1BR to antidepressant function as well
537 as to the behavioral and neural response to hallucinogens (62,63). As an inhibitory $G_{i/o}$-
538 coupled receptor, its activation reduces calcium influx in axon terminals of serotonin
539 (autoreceptors) and non-serotonin (heteroreceptors) neurons decreasing
540 neurotransmitter release (64–66). Past research shows that 5-HT1BR agonists promote
541 antidepressant-like effects and can increase the rewarding effects of cocaine and social
542 interaction (67–70). 5-HT1BR also interacts with p11 to regulate depression states
543 (71,72). Additionally, 5-HT1BR is necessary for the behavioral and hippocampal
544 plasticity effects of fluoxetine (33). In addition to mediating depressive phenotypes, there
545 is also evidence implicating the 5-HT1BR in antidepressant response to ketamine
546 (39,42), and in other behavioral effects of MDMA (38,40,41).

547 In keeping with our previous work showing that the 5-HT1BR has a known role in
548 mediating reward and anxiety behavior (36,43,58), we do find baseline differences in
549 some of the behaviors we tested in 5-HT1BR KO mice. Additionally, there were also
550 behavioral assays in which mice lacking 5-HT1BR seemed to show more resilience to
551 chronic corticosterone treatment, particularly in the gustometer measure of anhedonia.
552 These differences may arise from compensatory changes due to the absence of 5-
553 HT1BR throughout development. Alternatively, these baseline differences could be a
554 reflection of the important role of 5-HT1BR in mediating these phenotypes in adulthood.
555 However, the baseline differences leave open the limitation of potential behavioral and
556 mechanistic ceiling effects which could have obscured the effects of psilocybin in this
557 strain. This would be possible either through maxing out the range of the behavioral
558 assays and/or through saturation of the 5-HT1BR-dependent mechanism through which
559 psilocybin has its effects. We first addressed these limitations by using at least one
560 behavioral assay with no baseline differences, namely the elevated plus maze.
561 Additionally, we tested mice with adult knockdown of the receptor to eliminate any
562 developmental compensation. Finally, we used a pharmacological blockade of 5-HT1BR
563 by administering an antagonist prior to psilocybin administration. This not only allowed
564 us to circumvent any baseline differences, but also helped identify that 5-HT1BR is
565 involved during the acute drug effect.

566 Consistent with past research, we find some sex differences in our behavioral effects of
567 psilocybin. We found that the post-acute behavioral effects of psilocybin on anhedonia
568 and anxiety-like behavior were influenced by an interaction of stressor-type and sex.
569 Specifically, psilocybin was able to rescue the anhedonia and anxiety-like behavior in
570 females exposed to chronic corticosterone treatment, however, this effect was not seen
571 in males. This sex difference may have emerged from a differential response to the
572 stress paradigm rather than a differential response to psilocybin, particularly due to a
573 more robust effect of the corticosterone treatment on the behavior of males. This is
574 consistent with past work showing that there are sex-specific effects of chronic
575 corticosterone administration in the drinking water (73,74). On the other hand, using a
576 behavioral despair model via repeated forced swim stress, psilocybin was able to rescue
577 stress-induced behavioral phenotypes in both male and female mice. This suggests that
578 different stress modalities may induce sex-dependent alterations by engaging different

neural circuits that are amenable to varied pharmacological interventions. For example, recent work shows that psilocybin differentially influences hypothalamic reactivity to aversive stimuli and reduces ethanol consumption specifically in male, but not female rodents (59,75). On the other hand, females display more head twitch behavior to the psychedelic 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and less of an augmentation of pre-pulse inhibition in males, in a strain dependent manner (76,77). Ultimately, there appears to be a substantial impact of sex on the effect of psilocybin on behavior, which are dependent on strain and paradigm, reinforcing the importance of examining sex differences in future studies.

While the 5-HT2AR plays a central role in psilocybin's acute psychedelic effects, its therapeutic potential in the context of stress-induced depression may be limited without the support of other serotonin signaling pathways, including those acting via 5-HT1B. The absence of 5-HT1BR signaling may prevent psilocybin from achieving its full therapeutic potential as the feedback inhibition of serotonin release is disrupted, leading to overactivation of serotonergic circuits, and limiting the ability to modulate neural mechanisms effectively. This could result in a mechanistic ceiling of 5-HT2A, essentially maxing out the 5-HT2A-dependent effects on anhedonia and anxiety-like behaviors. This hypothesis arises in part from studies showing increased 5-HT2AR expression following chronic exposure to stress (78–80). However, neither stress nor the knock-out model seem to have affected acute 5-HT2AR activation in our study, as measured by head twitch responding, and past studies showing no effect of 5-HT1BR KO on 5-HT2AR binding (57). Additionally, although our hypothesis of 5-HT1BR involvement is via direct binding of psilocin to 5-HT1BRs on neural circuits implicated in anxiety and depressive behaviors, it is also possible that these effects may be indirect. For example, 5-HT1BR could influence the hypothermic response to psilocybin which could then influence the antidepressant-like behavioral response (81–83). Overall, in combination with other work, our results highlight that psilocybin's persisting effects on behavior may depend on an optimal balance of serotonin tone acting across different serotonin receptor subtypes potentially across a number of physiological systems.

Our studies begin to point to the anterior cingulate cortex, the claustrum, and the amygdala as potential targets of psilocybin which are modulated by 5-HT1BR expression. Some preclinical studies have already highlighted the amygdala, especially the basolateral and central nucleus of the amygdala, as important loci for the therapeutic effects of psilocybin (84–87). Given the role of the amygdala in mediating the effects of psilocybin on threat responding (88), it is possible that 5-HT1BR expression on amygdalar terminals underlies our reported behavioral effects. We also found that the 5-HT1BR-effects on functional connectivity seemed to involve the amygdala as a node given its high number of connections with other brain regions that were modulated by 5-HT1BR expression. Our current studies are focusing on region-specific knockdown of 5-HT1BRs in the amygdala to test their functional role in modulating the effects of psilocybin on emotional reactivity. Projection specific studies will also investigate if prefrontal-amygdala networks are involved, and how psilocybin may influence these connections via 5-HT1BR since serotonin is known to gate cortical glutamatergic inputs into the BLA via 5-HT1B receptors (89). Understanding how psilocybin affects interregional connectivity within these networks via its actions on 5-HT1BR activation will help identify the mechanisms through which psilocybin exerts its antidepressant effects.

625 Overall, our work highlights the complexity of the serotoninergic targets involved in the
626 therapeutic efficacy of psilocybin. Specifically, these studies add to our understanding of
627 the importance of non-5-HT2AR targets by implicating 5-HT1BRs in the antidepressant-
628 like effects of psilocybin in mice. These data contribute to the broader understanding of
629 the polypharmacology of psilocybin and highlight the need for multi-receptor approaches
630 to better understand psilocybin's mechanisms of action. Additionally, our data
631 demonstrate a dissociation of the acute head twitch response from the longer-term
632 effects of psilocybin, suggesting that different mechanisms of actions may underlie the
633 acute psychedelic effect of psilocybin and the persisting behavioral effects. We suggest
634 that psilocybin's effects are not solely driven by 5-HT2AR activation, and that activation
635 of other serotonin receptors are critical for its therapeutic potential. These results also
636 raise the potential for development of effective non-hallucinogenic pharmacotherapies
637 targeting 5-HT1BR for the treatment of depressive disorders.

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642 **References**
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644 1. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU. Twelve-month and
645 lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United
646 States. Int J Methods Psychiatr Res. 2012;21(3):169–84.

647 2. Gaynes BN, Warden D, Trivedi MH, Wisniewski SR, Fava M, Rush AJ. What did STAR*D teach
648 us? Results from a large-scale, practical, clinical trial for patients with depression. Psychiatr
649 Serv Wash DC. 2009 Nov;60(11):1439–45.

650 3. Carhart-Harris R, Giribaldi B, Watts R, Baker-Jones M, Murphy-Beiner A, Murphy R, et al.
651 Trial of Psilocybin versus Escitalopram for Depression. N Engl J Med. 2021 Apr
652 15;384(15):1402–11.

653 4. Carhart-Harris RL, Bolstridge M, Rucker J, Day CMJ, Erritzoe D, Kaelen M, et al. Psilocybin
654 with psychological support for treatment-resistant depression: an open-label feasibility
655 study. Lancet Psychiatry. 2016 Jul 1;3(7):619–27.

656 5. Davis AK, Barrett FS, May DG, Cosimano MP, Sepeda ND, Johnson MW, et al. Effects of
657 Psilocybin-Assisted Therapy on Major Depressive Disorder: A Randomized Clinical Trial.
658 JAMA Psychiatry. 2021 May 1;78(5):481.

659 6. Goldberg SB, Pace BT, Nicholas CR, Raison CL, Hutson PR. The experimental effects of
660 psilocybin on symptoms of anxiety and depression: A meta-analysis. Psychiatry Res. 2020
661 Feb;284:112749.

662 7. Goodwin GM, Aaronson ST, Alvarez O, Arden PC, Baker A, Bennett JC, et al. Single-Dose
663 Psilocybin for a Treatment-Resistant Episode of Major Depression. N Engl J Med. 2022 Nov
664 3;387(18):1637–48.

665 8. McClure-Begley TD, Roth BL. The promises and perils of psychedelic pharmacology for
666 psychiatry. Nat Rev Drug Discov. 2022 Jun;21(6):463–73.

667 9. Johnson MW, Griffiths RR. Potential Therapeutic Effects of Psilocybin. Neurotherapeutics.
668 2017 Jul 1;14(3):734–40.

669 10. Carhart-Harris RL, Bolstridge M, Day CMJ, Rucker J, Watts R, Erritzoe DE, et al. Psilocybin
670 with psychological support for treatment-resistant depression: six-month follow-up.
671 Psychopharmacology (Berl). 2018 Feb 1;235(2):399–408.

672 11. Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, et al.
673 Psilocybin produces substantial and sustained decreases in depression and anxiety in
674 patients with life-threatening cancer: A randomized double-blind trial. J Psychopharmacol
675 (Oxf). 2016 Dec 1;30(12):1181–97.

676 12. Gukasyan N, Davis AK, Barrett FS, Cosimano MP, Sepeda ND, Johnson MW, et al. Efficacy
677 and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-
678 month follow-up. J Psychopharmacol (Oxf). 2022 Feb 1;36(2):151–8.

679 13. Von Rotz R, Schindowski EM, Jungwirth J, Schuldt A, Rieser NM, Zahoranszky K, et al. Single-
680 dose psilocybin-assisted therapy in major depressive disorder: a placebo-controlled, double-
681 blind, randomised clinical trial. eClinicalMedicine. 2023 Feb;56:101809.

682 14. Raison CL, Sanacora G, Woolley J, Heinzerling K, Dunlop BW, Brown RT, et al. Single-Dose
683 Psilocybin Treatment for Major Depressive Disorder: A Randomized Clinical Trial. JAMA.
684 2023 Sep 5;330(9):843–53.

685 15. Wulff AB, Nichols CD, Thompson SM. Preclinical perspectives on the mechanisms underlying
686 the therapeutic actions of psilocybin in psychiatric disorders. Neuropharmacology. 2023 Jun
687 15;231:109504.

688 16. Chiu YT, Deutch AY, Wang W, Schmitz GP, Huang KL, Kocak DD, et al. A suite of engineered
689 mice for interrogating psychedelic drug actions. BioRxiv Prepr Serv Biol. 2023 Sep
690 26;2023.09.25.559347.

691 17. Madsen MK, Fisher PM, Burmester D, Dyssegaard A, Stenbæk DS, Kristiansen S, et al.
692 Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and
693 plasma psilocin levels. Neuropsychopharmacology. 2019 Jun;44(7):1328–34.

694 18. Roseman L, Nutt DJ, Carhart-Harris RL. Quality of Acute Psychedelic Experience Predicts
695 Therapeutic Efficacy of Psilocybin for Treatment-Resistant Depression. Front Pharmacol
696 [Internet]. 2018 Jan 17 [cited 2024 Oct 2];8. Available from:
697 https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2017.00974/ful
698 l

699 19. Yaden DB, Griffiths RR. The Subjective Effects of Psychedelics Are Necessary for Their
700 Enduring Therapeutic Effects. ACS Pharmacol Transl Sci. 2021 Apr 9;4(2):568–72.

701 20. Gumpper RH, Roth BL. SnapShot: Psychedelics and serotonin receptor signaling. Cell. 2023
702 Jan 5;186(1):232-232.e1.

703 21. Kim K, Che T, Panova O, DiBerto JF, Lyu J, Krumm BE, et al. Structure of a Hallucinogen-
704 Activated Gq-Coupled 5-HT2A Serotonin Receptor. Cell. 2020 Sep 17;182(6):1574-1588.e19.

705 22. Cameron LP, Patel SD, Vargas MV, Barragan EV, Saeger HN, Warren HT, et al. 5-HT2ARs
706 Mediate Therapeutic Behavioral Effects of Psychedelic Tryptamines. ACS Chem Neurosci.
707 2023 Feb 1;14(3):351–8.

708 23. Hesselgrave N, Troppoli TA, Wulff AB, Cole AB, Thompson SM. Harnessing psilocybin:
709 antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R
710 activation in mice. Proc Natl Acad Sci. 2021 Apr 27;118(17):e2022489118.

711 24. Hibicke M, Landry AN, Kramer HM, Talman ZK, Nichols CD. Psychedelics, but Not Ketamine,
712 Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the
713 Study of Depression. ACS Chem Neurosci. 2020 Mar 18;11(6):864–71.

714 25. Ly C, Greb AC, Cameron LP, Wong JM, Barragan EV, Wilson PC, et al. Psychedelics Promote
715 Structural and Functional Neural Plasticity. Cell Rep. 2018 Jun 12;23(11):3170–82.

716 26. Sekssaoui M, Bockaert J, Marin P, Bécamel C. Antidepressant-like effects of psychedelics in a
717 chronic despair mouse model: is the 5-HT2A receptor the unique player?
718 Neuropsychopharmacology. 2024 Jan 11;1–10.

719 27. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, et al. Transient Elevation of
720 Plasma Glucocorticoids Supports Psilocybin-Induced Anxiolysis in Mice. ACS Pharmacol
721 Transl Sci. 2023 Aug 11;6(8):1221–31.

722 28. Sekssaoui M, Bockaert J, Marin P, Bécamel C. Antidepressant-like effects of psychedelics in a
723 chronic despair mouse model: is the 5-HT2A receptor the unique player?
724 Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2024 Mar;49(4):747–56.

725 29. Kwan AC, Olson DE, Preller KH, Roth BL. The neural basis of psychedelic action. Nat
726 Neurosci. 2022 Nov;25(11):1407–19.

727 30. Shao LX, Liao C, Gregg I, Davoudian PA, Savalia NK, Delagarza K, et al. Psilocybin induces
728 rapid and persistent growth of dendritic spines in frontal cortex in vivo. Neuron. 2021 Aug
729 18;109(16):2535-2544.e4.

730 31. Moliner R, Girych M, Brunello CA, Kovaleva V, Biojone C, Enkavi G, et al. Psychedelics
731 promote plasticity by directly binding to BDNF receptor TrkB. Nat Neurosci. 2023
732 Jun;26(6):1032–41.

733 32. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of
734 indoleamine hallucinogens. Neuropharmacology. 2011 Sep 1;61(3):364–81.

735 33. Cai X, Kallarackal AJ, Kvarta MD, Goluskin S, Gaylor K, Bailey AM, et al. Local potentiation of
736 excitatory synapses by serotonin and its alteration in rodent models of depression. Nat
737 Neurosci. 2013 Apr;16(4):464–72.

738 34. Mathur BN, Capik NA, Alvarez VA, Lovinger DM. Serotonin Induces Long-Term Depression at
739 Corticostriatal Synapses. J Neurosci. 2011 May 18;31(20):7402–11.

740 35. Clark MS, Vincow ES, Sexton TJ, Neumaier JF. Increased expression of 5-HT1B receptor in
741 dorsal raphe nucleus decreases fear-potentiated startle in a stress dependent manner. Brain
742 Res. 2004 May 8;1007(1):86–97.

743 36. Nautiyal KM, Tritschler L, Ahmari SE, David DJ, Gardier AM, Hen R. A Lack of Serotonin 1B
744 Autoreceptors Results in Decreased Anxiety and Depression-Related Behaviors.
745 Neuropsychopharmacology. 2016 Nov;41(12):2941–50.

746 37. Neumaier JF, Vincow ES, Arvanitogiannis A, Wise RA, Carlezon WA. Elevated Expression of 5-
747 HT1B Receptors in Nucleus Accumbens Efferents Sensitizes Animals to Cocaine. J Neurosci.
748 2002 Dec 15;22(24):10856–63.

749 38. Aronsen D, Bukholt N, Schenk S. Repeated administration of the 5-$HT_1B/_1A$ agonist, RU
750 24969, facilitates the acquisition of MDMA self-administration: role of 5-HT_1A and 5-HT_1B
751 receptor mechanisms. Psychopharmacology (Berl). 2016 Apr;233(8):1339–47.

752 39. du Jardin KG, Liebenberg N, Cajina M, Müller HK, Elfving B, Sanchez C, et al. S-Ketamine
753 Mediates Its Acute and Sustained Antidepressant-Like Activity through a 5-HT1B Receptor
754 Dependent Mechanism in a Genetic Rat Model of Depression. Front Pharmacol [Internet].
755 2018 Jan 15 [cited 2024 Oct 2];8. Available from:
756 https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2017.00978/ful
757 l

758 40. Heifets BD, Salgado JS, Taylor MD, Hoerbelt P, Cardozo Pinto DF, Steinberg EE, et al. Distinct
759 neural mechanisms for the prosocial and rewarding properties of MDMA. Sci Transl Med.
760 2019 Dec 11;11(522):eaaw6435.

761 41. Scearce-Levie K, Viswanathan SS, Hen R. Locomotor response to MDMA is attenuated in
762 knockout mice lacking the 5-HT1B receptor. Psychopharmacology (Berl). 1999
763 Jan;141(2):154–61.

764 42. Yamanaka H, Yokoyama C, Mizuma H, Kurai S, Finnema SJ, Halldin C, et al. A possible
765 mechanism of the nucleus accumbens and ventral pallidum 5-HT1B receptors underlying
766 the antidepressant action of ketamine: a PET study with macaques. Transl Psychiatry. 2014
767 Jan;4(1):e342–e342.

768 43. Nautiyal KM, Tanaka KF, Barr MM, Tritschler L, Le Dantec Y, David DJ, et al. Distinct Circuits
769 Underlie the Effects of 5-HT1B Receptors on Aggression and Impulsivity. Neuron. 2015
770 May;86(3):813–26.

771 44. Harari R, Chatterjee I, Getselter D, Elliott E. Psilocybin induces acute anxiety and changes in
772 amygdalar phosphopeptides independently from the 5-HT2A receptor. iScience [Internet].
773 2024 May 17 [cited 2025 May 29];27(5). Available from:
774 https://www.cell.com/iscience/abstract/S2589-0042(24)00908-8

775 45. Farinha-Ferreira M, Miranda-Lourenço C, Galipeau C, Lenkei Z, Sebastião AM. Concurrent
776 stress modulates the acute and post-acute effects of psilocybin in a sex-dependent manner.
777 Neuropharmacology. 2025 Mar 15;266:110280.

778 46. Singh S, Botvinnik A, Shahar O, Wolf G, Yakobi C, Saban M, et al. Effect of psilocybin on
779 marble burying in ICR mice: role of 5-HT1A receptors and implications for the treatment of
780 obsessive-compulsive disorder. Transl Psychiatry. 2023 May 10;13(1):1–7.

781 47. de Almeida RM, Nikulina EM, Faccidomo S, Fish EW, Miczek KA. Zolmitriptan – a 5-HT1B/D
782 agonist, alcohol, and aggression in mice. Psychopharmacology (Berl). 2001 Sep
783 1;157(2):131–41.

784 48. Conductier G, Crosson C, Hen R, Bockaert J, Compan V. 3,4-N-
785 Methlenedioxymethamphetamine-Induced Hypophagia is Maintained in 5-HT1B Receptor
786 Knockout Mice, but Suppressed by the 5-HT2C Receptor Antagonist RS102221.
787 Neuropsychopharmacology. 2005 Jun;30(6):1056–63.

788 49. Skingle M, Beattie DT, Scopes DIC, Starkey SJ, Connor HE, Feniuk W, et al. GR127935: a
789 potent and selective 5-HT1D receptor antagonist. Behav Brain Res. 1995 Dec 15;73(1):157–
790 61.

791 50. De Vries P, Apaydin S, Villalón CM, Heiligers JP, Saxena PR. Interactions of GR127935, a 5-
792 HT(1B/D) receptor ligand, with functional 5-HT receptors. Naunyn Schmiedebergs Arch
793 Pharmacol. 1997 Apr;355(4):423–30.

794 51. Dieterich A, Stech K, Srivastava P, Lee J, Sharif A, Samuels BA. Chronic corticosterone shifts
795 effort-related choice behavior in male mice. Psychopharmacology (Berl). 2020 Jul
796 1;237(7):2103–10.

797 52. Terstege DJ, Oboh DO, Epp JR. FASTMAP: Open-Source Flexible Atlas Segmentation Tool for
798 Multi-Area Processing of Biological Images. eNeuro [Internet]. 2022 Apr [cited 2023 May
799 26];9(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8938980/

800 53. Castro ME, Pascual J, Romón T, Berciano J, Figols J, Pazos A. 5-HT1B receptor binding in
801 degenerative movement disorders. Brain Res. 1998 Apr 20;790(1–2):323–8.

802 54. Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, et al.
803 Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in
804 rodent and primate brain. Neuropharmacology. 1994;33(3–4):367–86.

805 55. González-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, et al. Hallucinogens recruit
806 specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron.
807 2007 Feb 1;53(3):439–52.

808 56. Halberstadt AL, Geyer MA. Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine
809 (2C-I) and superpotent N-benzyl derivatives on the head twitch response.
810 Neuropharmacology. 2014 Feb;77:200–7.

811 57. Lee MD, Somerville EM, Kennett GA, Dourish CT, Clifton PG. Reduced hypophagic effects of
812 d-fenfluramine and the 5-HT2C receptor agonist mCPP in 5-HT1B receptor knockout mice.
813 Psychopharmacology (Berl). 2004 Oct 1;176(1):39–49.

814 58. Desrochers SS, Lesko EK, Magalong VM, Balsam PD, Nautiyal KM. A role for reward valuation
815 in the serotonergic modulation of impulsivity. Psychopharmacology (Berl). 2021 Nov
816 1;238(11):3293–309.

817 59. Effinger DP, Hoffman JL, Mott SE, Magee SN, Quadir SG, Rollison CS, et al. Increased
818 reactivity of the paraventricular nucleus of the hypothalamus and decreased threat
819 responding in male rats following psilocin administration. Nat Commun. 2024 Jun
820 22;15:5321.

821 60. Rijsketic DR, Casey AB, Barbosa DAN, Zhang X, Hietamies TM, Ramirez-Ovalle G, et al.
822 UNRAVELing the synergistic effects of psilocybin and environment on brain-wide immediate
823 early gene expression in mice. Neuropsychopharmacol Off Publ Am Coll
824 Neuropsychopharmacol. 2023 Nov;48(12):1798–807.

825 61. Davoudian PA, Shao LX, Kwan AC. Shared and Distinct Brain Regions Targeted for Immediate
826 Early Gene Expression by Ketamine and Psilocybin. ACS Chem Neurosci. 2023 Feb
827 1;14(3):468–80.

828 62. Nautiyal KM, Hen R. Serotonin receptors in depression: from A to B. F1000Research. 2017
829 Feb 9;6:123.

830 63. Tiger M, Varnäs K, Okubo Y, Lundberg J. The 5-HT1B receptor - a potential target for
831 antidepressant treatment. Psychopharmacology (Berl). 2018;235(5):1317–34.

832 64. Boschert U, Aït. Amara D, Segu L, Hen R. The mouse 5-hydroxytryptamine 1B receptor is
833 localized predominantly on axon terminals. Neuroscience. 1994 Jan 1;58(1):167–82.

834 65. Sari Y. Serotonin1B receptors: from protein to physiological function and behavior. Neurosci
835 Biobehav Rev. 2004 Oct;28(6):565–82.

836 66. Mizutani H, Hori T, Takahashi T. 5-HT $_{1B}$ receptor-mediated presynaptic inhibition at the
837 calyx of Held of immature rats. Eur J Neurosci. 2006 Oct;24(7):1946–54.

838 67. Chenu F, David DJP, Leroux-Nicollet I, Le Maître E, Gardier AM, Bourin M. Serotonin1B
839 heteroreceptor activation induces an antidepressant-like effect in mice with an alteration of
840 the serotonergic system. J Psychiatry Neurosci JPN. 2008 Nov;33(6):541–50.

841 68. Dölen G, Darvishzadeh A, Huang KW, Malenka RC. Social reward requires coordinated
842 activity of nucleus accumbens oxytocin and serotonin. Nature. 2013 Sep;501(7466):179–84.

843 69. Pentkowski NS, Acosta JI, Browning JR, Hamilton EC, Neisewander JL. PRECLINICAL STUDY:
844 Stimulation of 5-HT1B receptors enhances cocaine reinforcement yet reduces cocaine-
845 seeking behavior. Addict Biol. 2009;14(4):419–30.

846 70. Tatarczynska E, Klodzinska A, Stachowicz K, Chojnacka-Wójcik E. Effects of a selective 5-
847 HT1B receptor agonist and antagonists in animal models of anxiety and depression. Behav
848 Pharmacol. 2004 Dec;15(8):523.

849 71. Alexander B, Warner-Schmidt J, Eriksson T, Tamminga C, Arango-Lievano M, Ghose S, et al.
850 Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens. Sci
851 Transl Med. 2010 Oct 20;2(54):54ra76.

852 72. Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, Yacoubi ME, et al. Alterations in
853 5-HT1B Receptor Function by p11 in Depression-Like States. Science. 2006 Jan
854 6;311(5757):77–80.

855 73. Berger S, Gureczny S, Reisinger SN, Horvath O, Pollak DD. Effect of Chronic Corticosterone
856 Treatment on Depression-Like Behavior and Sociability in Female and Male C57BL/6N Mice.
857 Cells. 2019 Sep;8(9):1018.

858 74. Mekiri M, Gardier AM, David DJ, Guilloux JP. Chronic corticosterone administration effects
859 on behavioral emotionality in female c57bl6 mice. Exp Clin Psychopharmacol. 2017
860 Apr;25(2):94–104.

861 75. Alper K, Cange J, Sah R, Schreiber-Gregory D, Sershen H, Vinod KY. Psilocybin sex-
862 dependently reduces alcohol consumption in C57BL/6J mice. Front Pharmacol.
863 2022;13:1074633.

864 76. Jaster AM, Younkin J, Cuddy T, de la Fuente Revenga M, Poklis JL, Dozmorov MG, et al.
865 Differences across sexes on head-twitch behavior and 5-HT2A receptor signaling in C57BL/6J
866 mice. Neurosci Lett. 2022 Sep 25;788:136836.

867 77. Vohra HZ, Saunders JM, Jaster AM, de la Fuente Revenga M, Jimenez J, Fernández-Teruel A,
868 et al. Sex-specific effects of psychedelics on prepulse inhibition of startle in 129S6/SvEv
869 mice. Psychopharmacology (Berl). 2022 Jun;239(6):1649–64.

870 78. Trajkovska V, Kirkegaard L, Krey G, Marcussen AB, Thomsen MS, Chourbaji S, et al.
871 Activation of glucocorticoid receptors increases 5-HT2A receptor levels. Exp Neurol. 2009 Jul
872 1;218(1):83–91.

873 79. Dwivedi Y, Mondal AC, Payappagoudar GV, Rizavi HS. Differential regulation of serotonin
874 (5HT)2A receptor mRNA and protein levels after single and repeated stress in rat brain: role
875 in learned helplessness behavior. Neuropharmacology. 2005 Feb 1;48(2):204–14.

80. Schiller L, Jähkel M, Kretzschmar M, Brust P, Oehler J. Autoradiographic analyses of 5-HT1A and 5-HT2A receptors after social isolation in mice. Brain Res. 2003 Aug 8;980(2):169–78.

81. Alitalo O, Kohtala S, Rosenholm M, Saarreharju R, González-Hernández G, Sarparanta M, et al. Nitrous oxide induces hypothermia and TrkB activation: Maintenance of body temperature abolishes antidepressant-like effects in mice. Neuropharmacology. 2024 Dec 15;261:110172.

82. Oerther S, Ahlenius S. Involvement of 5-HT1A and 5-HT1B receptors for citalopram-induced hypothermia in the rat. Psychopharmacology (Berl). 2001 Apr 1;154(4):429–34.

83. McDougall SA, Roe MJ, Robinson JAM, Cotter LL, Gonzalez DJ, Gleason DC, et al. Effects of the serotonin 5-HT1B receptor agonist CP 94253 on the locomotor activity and body temperature of preweanling and adult male and female rats. Eur J Pharmacol. 2022 Jul 5;926:175019.

84. Effinger DP, Quadir SG, Ramage MC, Cone MG, Herman MA. Sex-specific effects of psychedelic drug exposure on central amygdala reactivity and behavioral responding. Transl Psychiatry. 2023 Apr 8;13(1):1–15.

85. Funk D, Araujo J, Slassi M, Lanthier J, Atkinson J, Feng D, et al. Effect of a single psilocybin treatment on Fos protein expression in male rat brain. Neuroscience. 2024 Feb 16;539:1–11.

86. Harari R, Chatterjee I, Getselter D, Elliott E. Psilocybin induces acute anxiety and changes in amygdalar phosphopeptides independently from the 5-HT2A receptor. iScience. 2024 May;27(5):109686.

87. Mertens LJ, Wall MB, Roseman L, Demetriou L, Nutt DJ, Carhart-Harris RL. Therapeutic mechanisms of psilocybin: Changes in amygdala and prefrontal functional connectivity during emotional processing after psilocybin for treatment-resistant depression. J Psychopharmacol (Oxf). 2020 Feb 1;34(2):167–80.

88. Effinger DP, Quadir SG, Ramage MC, Cone MG, Herman MA. Sex-specific effects of psychedelic drug exposure on central amygdala reactivity and behavioral responding. Transl Psychiatry. 2023 Apr 8;13(1):119.

89. Guo JD, O'Flaherty BM, Rainnie DG. Serotonin gating of cortical and thalamic glutamate inputs onto principal neurons of the basolateral amygdala. Neuropharmacology. 2017 Nov;126:224–32.

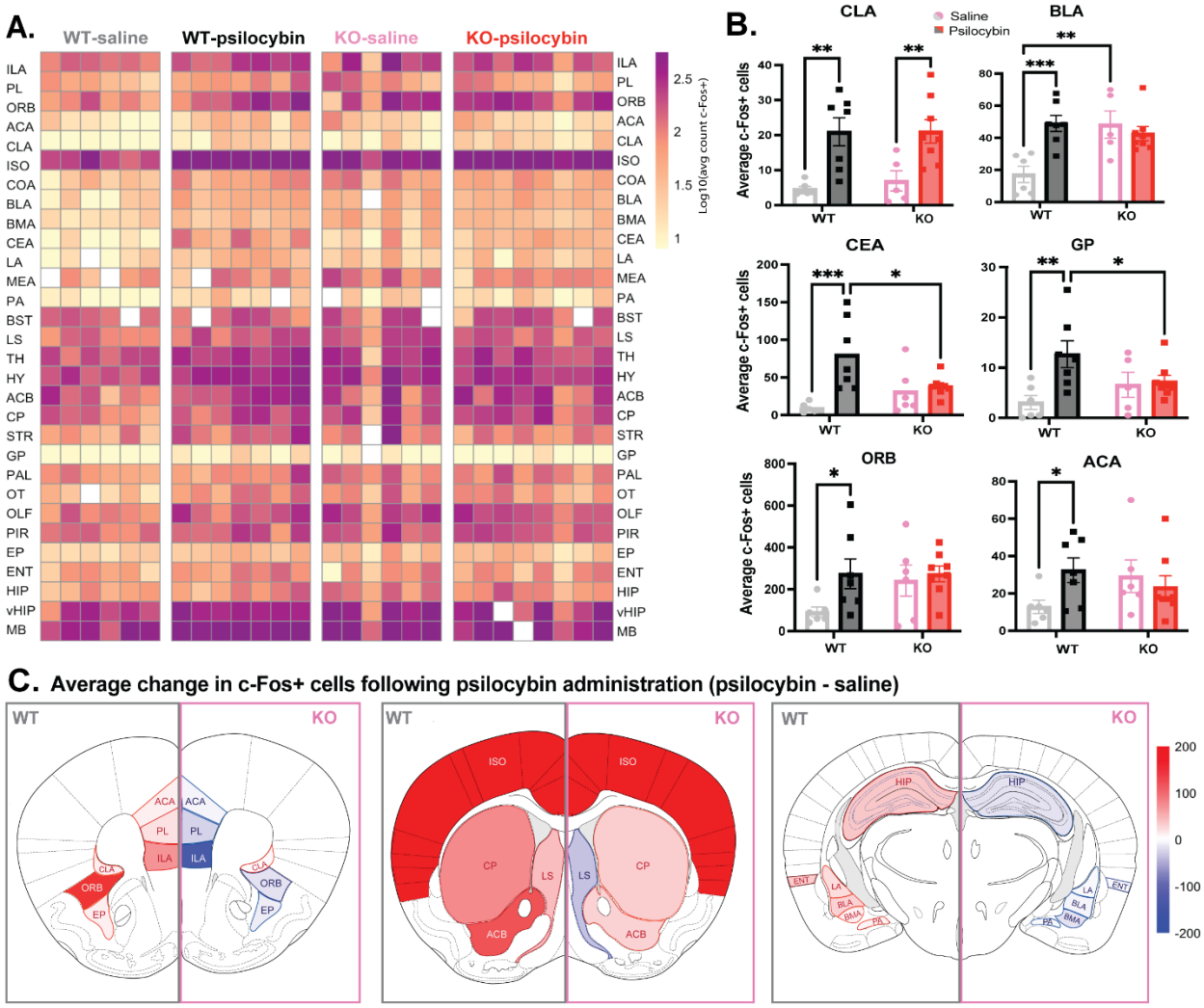

Figure 1: Psilocybin-induced changes in neural activity are dependent on 5-HT1BR
(A) Heatmap shows log-transformed counts of c-Fos+ cells for individual mice (columns) across brain regions (rows) for control and 5-HT1BR knockout (KO) mice treated with saline or psilocybin. White squares indicate missing data points. **(B)** Group means of c-Fos+ cells in six representative brain regions following psilocybin or saline treatment. **(C)** Percent change in c-Fos+ cells following psilocybin administration is shown in three diagrams of coronal sections of the brain (at bregma +1.94mm, +0.98mm, and -2.18mm) in control (left hemisphere of each diagram) and 5-HT1BR knockout (right hemisphere of each diagram) mice. Psilocybin leads to both increases (shown in red) and decreases (blue) in c-Fos+ cell counts, with notable differences between genotypes, calculated as relative changes in c-Fos expression compared to saline-injected controls, with increases colored as red if they were greater than 200%. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ILA: infralimbic cortex, PL: prelimbic cortex; ORB: orbitofrontal cortex, ACA: anterior cingulate cortex, CLA: claustrum, ISO: isocortex, COA: cortical amygdala, BLA: basolateral amygdala, BMA: basomedial amygdala, CEA: central amygdala, LA: lateral amygdala, MEA: medial amygdala, PA: posterior amygdala nucleus, BST: bed nucleus of the stria terminalis, LS: lateral septum, TH: thalamus, HY: hypothalamus, ACB: nucleus accumbens, CP: caudate putamen, STR: dorsal striatum, GP: globus pallidus, PAL: pallidum, OT: olfactory tubercle, OLF: olfactory areas, PIR: piriform cortex, EP: endopiriform nucleus, ENT: entorhinal area, dHIP: dorsal hippocampus, vHIP: ventral hippocampus, MB: midbrain

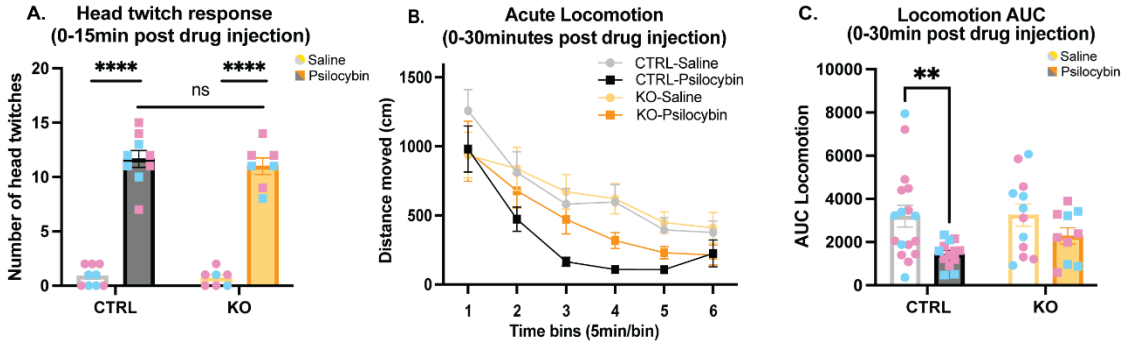

Figure 2: 5-HT1BR may be involved in some of the acute effects of psilocybin

(A) Head twitch response is shown for 5-HT1BR KO mice and littermate controls over the 15-minute period following administration of saline or psilocybin. Psilocybin treatment significantly increased the number of head twitches in both controls and KO mice compared to saline-treated controls with no significant difference between genotypes. (B) Locomotor activity is shown for 5-HT1BR KO mice and littermate controls mice over the 30 minutes following administration of saline or psilocybin. (C) Area under the curve of the locomotor activity over the 30 min following treatment summarizes the locomotor effects of psilocybin. Individual animals are shown as circles (saline) or squares (psilocybin) and colored by sex (pink for females, blue for males). Group data is represented as mean ± SEM. *, $p < 0.05$; ****$p < 0.0001$.

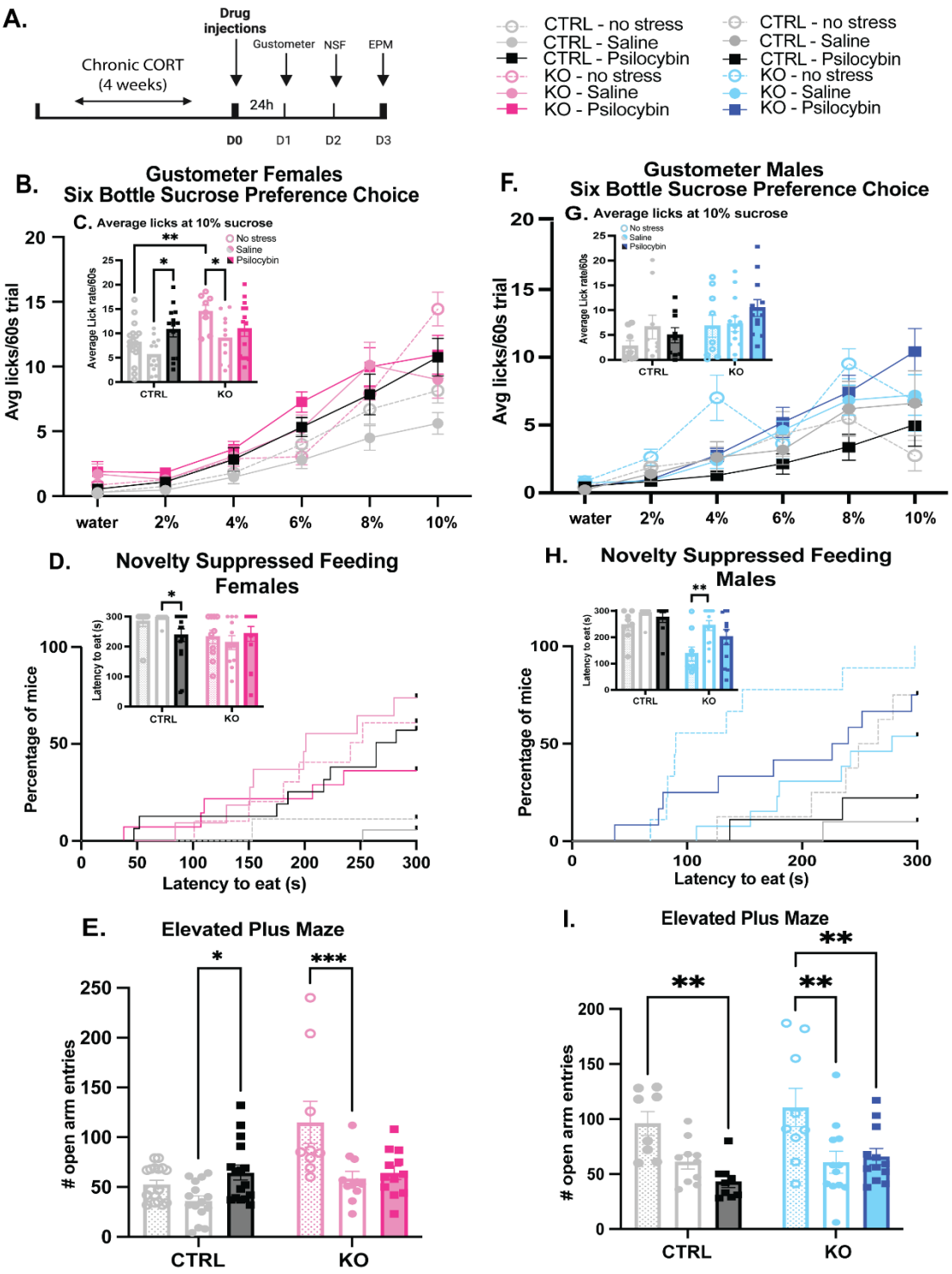

Figure 3: 5-HT1BR is required for behavioral effects of psilocybin in female mice.

(A) Experimental timeline is shown for studies testing the post-acute behavioral effects of psilocybin. **(B)** Gustometer data shows the number of licks in females across 6 concentrations of sucrose in female control and 5-HT1BR KO mice, following chronic cort with saline or psilocybin treatment, and for the no stress control condition. **(C)** Inset shows the number of licks at the 10% sucrose concentration in females. **(D)** Kaplan-Meier survival curves represent the cumulative

955 proportion of females eating over time in the NSF for controls (shades of black) and 5-HT1BR
956 KOs (shades of pink). Inset shows individual data points and average group latency to eat. **(E)**
957 Open arm entries are shown for females in the EPM for controls and KOs over the three
958 treatment groups. **(F)** Gustometer data shows the number of licks in males across 6
959 concentrations of sucrose in female control and 5-HT1BR KO mice, following chronic cort with
960 saline or psilocybin treatment, and for the no stress control condition. **(G)** Inset shows the number
961 of licks at the 10% sucrose concentration in males. **(H)** Kaplan-Meier survival curves represent
962 the cumulative proportion of males eating over time in the NSF for controls (shades of black) and
963 5-HT1BR KOs (shades of pink). Inset shows individual data points and average group latency to
964 eat. **(I)** Open arm entries are shown for males in the EPM for controls and KOs over the three
965 treatment groups. Data points on bar graphs represent individual mice and group averages are
966 shown as mean ± SEM. *p<0.05, **p<0.005, ***p<0.001

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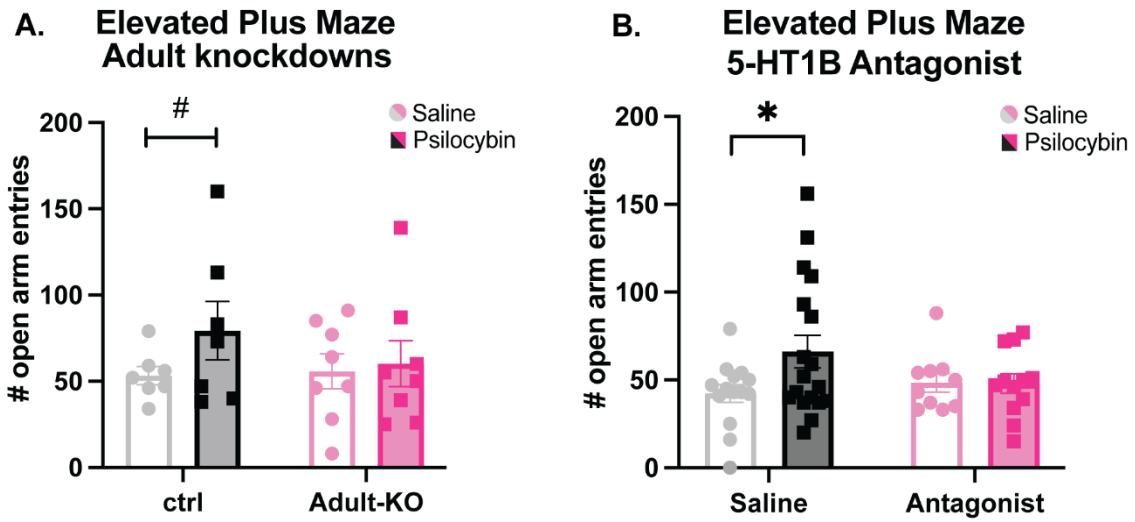

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970 **Figure 4: (A)** There was no significant effect of psilocybin in mice with adult knockout of 5-
971 HT1BR expression, however the effect of psilocybin in control mice did not reach statistical
972 significance in this cohort either. **(B)** Psilocybin significantly increased entries into the open arms
973 of the EPM in saline-treated controls mice, suggesting reduced anxiety. However, pre-treatment
974 with the 5-HT1BR antagonist GR127935 blocked this effect, indicating the importance of 5-
975 HT1BR activation during the acute phase of psilocybin for its anxiolytic effects. #p=0.1; *p < 0.05

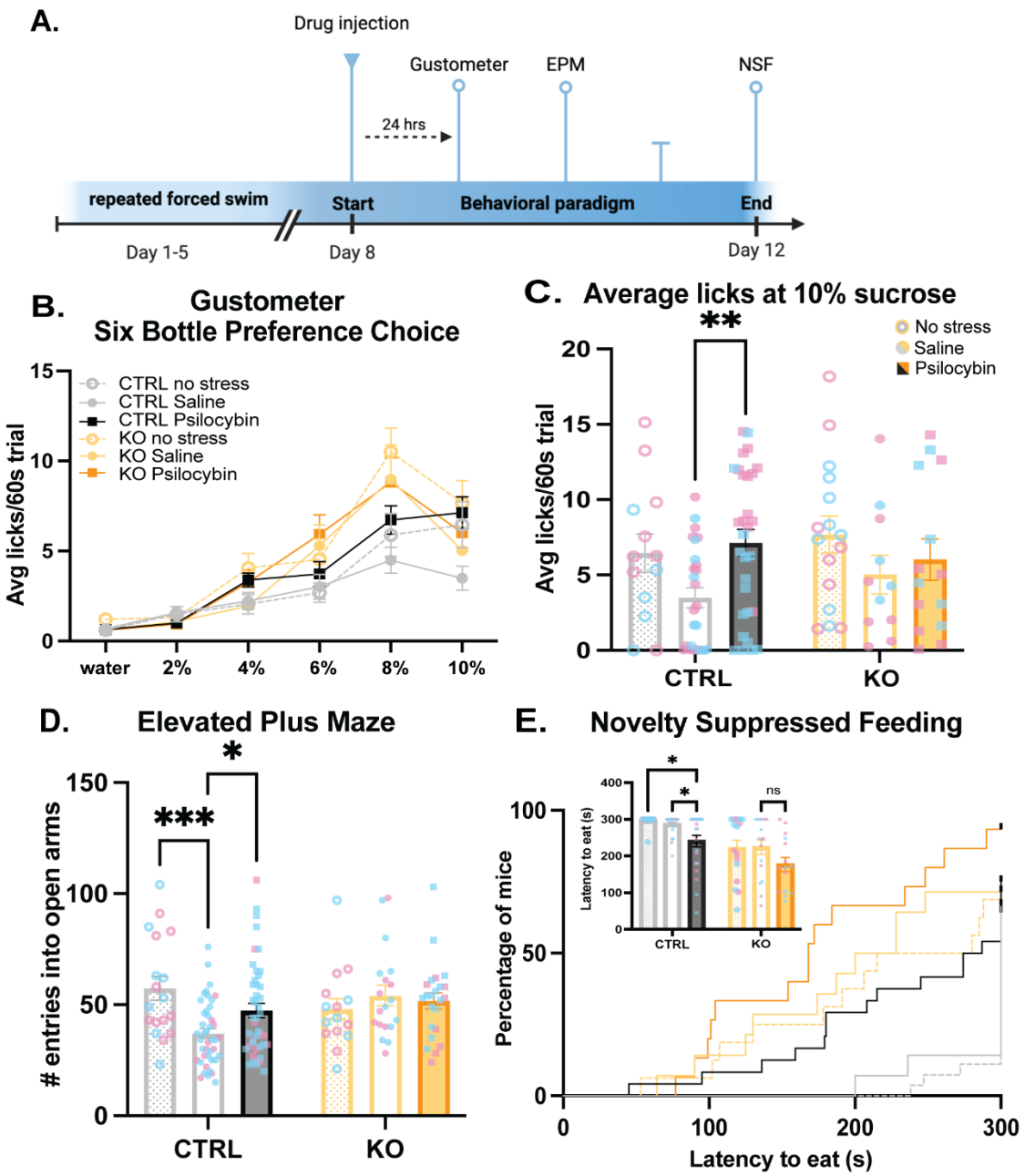

Figure 5. The effects of Psilocybin on behavior in a behavioral despair model are mediated by 5-HT1BR expression in both male and female mice. (A) Experimental timeline is shown for testing the post-acute behavioral effects of psilocybin following forced swim stress exposure. **(B)** Gustometer data shows the number of licks across 6 concentrations of sucrose in control and 5-HT1BR KO mice, following forced swim stress with saline or psilocybin treatment, and for the no stress control condition. **(C)** The number of licks at the 10% sucrose concentration is shown for control and 5-HT1BR KO mice in the three treatment conditions. **(D)** Open arm entries are shown in the EPM for both sexes in control and 5-HT1BR KO mice in three treatment groups. **(E)** Kaplan-Meier survival curves represent the cumulative proportion of mice eating over time in the NSF for controls (shades of black) and 5-HT1BR KOs (shades of yellow). Inset shows individual data points and average group latency to eat. Data points on bar graphs represent individual mice, with females colored pink and males colored blue. Group averages are shown as mean ± SEM. *p<0.05, **p<0.005, ***p<0.001

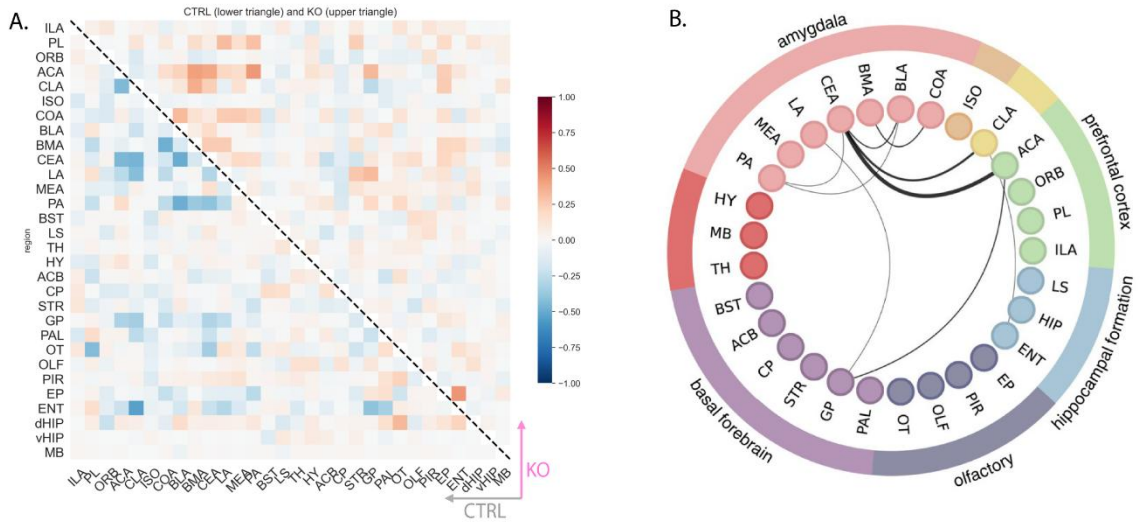

Figure 6: 5-HT1BR expression influences select neural circuits influenced by psilocybin

(A) The difference correlation matrix shows psilocybin-induced alterations in functional connectivity between control (lower triangle) and 5-HT1BR KO mice (upper triangle). The matrix represents the pairwise correlation differences across 30 regions, illustrating desynchrony patterns between the two genotypes, with control mice exhibiting more desynchrony following psilocybin than 5-HT1BR KO mice do. (B) Circular network plot highlighting psilocybin-responsive connections most influenced by 5-HT1BR expression. Significant changes in correlated activity were observed between the CEA and the ACA ($p = 0.05$). Expanding the confidence interval to 90%, 85%, and 80% revealed additional differences in connectivity in CEA-CLA, ACA-GP, CEA-COA, CEA-BLA, CLA-ENT, GP-LA, PA-CEA, PA-BLA. Line width corresponds to the confidence interval, with the thickest edge representing 95% CI.